IB04/03063

CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 19 September 2003 with an application for Letters Patent number 528388 made by EndocrinZ Ltd.

I further certify that pursuant to a claim under Section 24(1) of the Patents Act 1953, a direction was given that the application proceed in the name of Neuren Pharmaceuticals Limited.

Dated 3 September 2004

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NEW ZEALAND

528388

Patents Form No. 4 Our Ref: 1007/2

Patents Act 1953
PROVISIONAL SPECIFICATION

ENHANCED METHOD OF TREATMENT OF GROWTH DISORDERS

We, EndocrinZ Ltd. of Level 2, 2-6 Park Avenue, Grafton, Auckland do hereby declare this invention to be described in the following statement.

ENHANCED METHOD OF TREATMENT OF GROWTH DISORDERS

FIELD OF INVENTION

The invention pertains to conditions and diseases for which growth hormone is a desirable method of treatment. In particular, the present invention discloses an enhanced method of treatment of growth disorders.

BACKGROUND

GH therapy is used in the treatment of a variety of conditions. However, conventional GH therapy is subject to the presence of detrimental side effects. Side effects of GH therapy include glucose intolerance and/or diabetes, oedema, benign intracranial hypertension, arthralgia, myalgia, paresthesias and carpal tunnel syndrome. Oedema is defined as an accumulation of an excessive amount of watery fluid in cells, tissues or serous cavities (such as the abdomen). Symptoms include puffiness of the face around the eyes, or in the feet, ankles and legs. GH induced salt and water retention can cause benign intracranial hypertension. Benign intracranial hypertension is characterized by increased cerebrospinal fluid pressure in the absence of a space occupying lesion. It can present with headache, visual loss, nausea, vomiting and papilloedema. Arthralgia is pain in one or more joints. Myalgia is pain or discomfort moving any muscle(s). Paresthesia is a term that refers to an abnormal burning or prickling sensation which is generally felt in the hands, arms, legs, or feet, but can occur in any part of the body. Carpal tunnel syndrome occurs when tendons or ligaments in the wrist become enlarged, often from inflammation. The narrowed tunnel of bones and ligaments in the wrist pinches the nerves that reach the fingers and the muscles at the base of the thumb. Symptoms range from a burning, tingling numbness in the fingers, especially the thumb and the index and middle fingers, to difficulty gripping or making a fist, to dropping things.

There has been some concern about the possibility of "cancer growth promotion" with growth hormone therapy, based upon a few cases of leukaemia reported in children treated with growth hormone therapy.

Growth hormone is known to antagonise the actions of insulin through multiple steps in the insulin-signalling cascade. GH therapy has been shown to impair insulin-mediated suppression of hepatic glucose output and increased peripheral glucose utilization (Sugimoto *et al* 1998). Some of the insulin antagonistic effects of GH are thought to be due

to increased lipolysis and subsequent elevation in plasma FFA leading to inhibition of glucose uptake (Moller *et al* 1987). An increase in circulating FFA is associated with a reduction in insulin sensitivity as FFAs are known to impair insulin mediated glucose uptake in skeletal muscle (Felber *et al* 1964, Reaven *et al* 1988, Randle *et al* 1963).

The diabetogenic effects of GH therapy during childhood have recently been highlighted. An increased incidence of type 2 diabetes mellitus in children and adolescents during GH therapy has been found in populations at greatest risk of the disease (Cutfield *et al* 2000). Adult males born of low birth weight have an increased incidence of type 2 diabetes mellitus, dyslipidemia and hypertension (Barker *et al* 1993, Barker 1994, Law *et al* 1991).

It has been shown that prepubertal short IUGR children have markedly reduced insulin sensitivity, i.e. they are insulin resistant, compared to short children of normal birth weight (Hofman *et al* 1997). Girls with Turner syndrome have also been shown to exhibit reduced insulin sensitivity when compared to normal girls (Caprio *et al* 1991).

Reduced insulin sensitivity or secondary hyperinsulinism has been implicated in the pathogenesis of all the above mentioned disorders (Reaven *et al* 1991). Insulin resistance has been found to be a marker of type 2 diabetes mellitus in those at risk of type 2 diabetes (Martin *et al* 1992). In non-diabetic, euglycemic humans and animals fasting hyperinsulinemia reflects a generalised increase in insulin secretion that is a compensatory response for a reduction in insulin sensitivity (Kahn *et al* 1993). In addition, insulin resistance is involved in the pathogenesis of hypertension.

Insulin resistance and secondary hyperinsulinism are important in the pathogenesis of hypertension which occurs more commonly in adults of low birth weight (Barker *et al* 1993, Law *et al* 1991). Insulin has an important vasodilatory function that is mediated through nitric oxide release (McNally *et al* 1995, Steinberg *et al* 1994). Insulin-induced vasodilation is impaired in disorders characterised by insulin resistance (Laakso *et al* 1992, Laakso *et al* 1993, Feldman *et al* 1993).).

The applicants have previously observed that in IUGR children the marked reduction in insulin sensitivity that occurred during GH therapy was still present 3 months after stopping treatment (Cutfield, *et al* 2000 (2)).

In the light of the above observations it is clearly advantageous to establish a method of eliminating or at least alleviating the side effects of the GH treatment of growth disorders.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a method of preventing or treating the adverse consequences of growth hormone treatment of a growth disorder in a mammal, comprising administering an effective amount of a FFA regulator or a combination of FFA regulators prior to, in combination with or following the growth hormone treatment.

In further aspect the invention provides a method of treating growth disorders in a mammal comprising administering an effective amount of a FFA regulator or a combination of FFA regulators in combination with the growth hormone treatment.

In yet further aspect, this invention includes compositions suitable for the practice of the methods of the first two aspects of the invention.

DETAILED DESCRIPTION OF FIGURES

FIG. 1a and 1b shows weight gain curves of AD and UN animals during treatment..

FIG. 2a and 2b show anus to nose lengths of the AD and UN groups post-mortem.

FIG. 3 shows the effects of the treatments in AD and UN groups on blood haematocrit.

FIG. 4 shows liver weights as a percentage of a total body weight.

FIG. 5 shows retroperitoneal fat mass as a percentage of total body weight.

FIG. 6 shows adrenal weights as a percentage of total body weight.

FIG. 7 shows spleen weights as a percentage of total body weight.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the term 'growth hormone' or 'GH', in the context of the present application, includes growth hormone; growth hormone secretagogues (GHSs); growth hormone releasing proteins/peptides (GHRP); growth hormone releasing hormone (GHRH); somatotropin release inhibitory factor (SRIF); compounds which increase the endogenous release of growth hormone or growth hormone secretagogues; a pharmaceutically acceptable salt of a GHS; analogues; mimetics; functionally equivalent ligands; prodrugs; metabolites; derivatives; agonists; compounds which increase the activity of neural growth hormone receptors; compounds which bind to or increase the concentration of compounds which bind to neural growth hormone receptors; compounds which lessen or prevent inhibition of GH, GHS or ligand activity; or inhibitors of antagonists thereof.

Examples of agents which stimulate growth hormone and production or lessen or prevent its inhibition include, but are not limited to, growth hormone releasing peptides such as GHRP-1, GHRP-2 (also known as KP-102), GHRP-6, hexarelin, G-7039, G-7502, L-692,429, L-629,585, L-163,191 (aka MK-0677), ipamorelin, NN703, GHS-25, CP-424,391, ghrelin, SM-130686 or GHRH or inhibitors of GH antagonists (substances which bind growth hormone or otherwise prevent or reduce the action of GH within the body). These latter compounds exert an indirect effect on effective GH concentrations through the removal of an inhibitory mechanism and include substances such as somatostatin release inhibitory factor (SRIF).

The GH can be any GH in native-sequence or in variant form and from any source, whether natural, synthetic or recombinant. Examples being human GH, bovine GH, rat GH and porcine GH. It is, however, preferred that the GH employed be human GH.

As used herein, the term 'insulin resistance' refers to any condition or state whereby the actions of insulin are impaired or reduced.

As used herein, the term 'hyperinsulinemia' refers to the production of excessive amounts of insulin.

As used herein "treatment" of a disease or "therapy" for it includes preventing the disease from occurring in a mammal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment), inhibiting the disease (slowing or arresting its development), providing relief from the symptoms or side effects of the disease (including palliative treatment), and relieving the disease (causing regression of the disease).

As used herein, the term FFA regulator refers to any compound that has a hypolipidemic effect *i.e.* lowers FFA levels. The effects of fibrates are mediated by activation of peroxisome proliferators-activated receptors (PPAR). PPARα is thought to mediate the hypotriglyceridemic effect of fibrates by stimulating catabolic pathways of fatty acids in the liver. PPARα activators also decrease adipose tissue mass. Fenofibrate, ciprofibrate and GW9578 have been found to reduce insulin resistance without adverse effects on body weight and adipose tissue mass in an animal model. PPARα agonists may exert direct insulin-sensitising actions. Bezafibrate has been shown to reduce fat deposits and improve insulin sensitivity. The FFA regulators of interest include but are not limited to

fibric acid and nicotinic acid derivatives. The fibric acid derivatives include, but are not limited to, fenofibrate, clofibrate, gemfibrozil, bezafibrate and ciprofibrate. The nicotinic acid derivatives include but are not limited to acipimox.

Conditions treated using GH

As used herein, the term 'growth disorder' refers to any condition resulting in short stature. Such conditions include but are not limited to growth hormone insufficiency, growth hormone deficiency, intrauterine growth retardation, prematurity, skeletal dysplasias, chromosomal variations (Turner's Syndrome, Down Syndrome, Prader-Willi Syndrome), or chronic renal insufficiency related growth retardation, constitutional delay of growth, cystic fibrosis related growth retardation, or any other condition resulting in short stature.

GH Deficiency

Diagnosis of growth hormone deficiency requires growth hormone stimulation testing. Tests used include the insulin hypoglycemia test or insulin tolerance test (ITT), L-dopa stimulation test, arginine infusion test and arginine/GHRH test. Peak growth hormone secretion levels in adults of less than 3-5 ng/mL are indicative of GHD. In children values below 10 ng/mL are considered inadequate. Growth hormone deficiency is treated with recombinant human growth hormone which is usually given *via* a subcutaneous injection on a daily basis.

There are several causes of GHD in children and most can be related to a problem in the hypothalamus or the pituitary. In certain rare cases, a defect in the body's utilization of growth hormone occurs. In most children with growth hormone deficiency, the defect lies in the hypothalamus. When other pituitary hormones are also not being secreted normally, the child is said to have hypopituitarism. In congenital hypopituitarism, abnormal formation of the pituitary or hypothalamus occurs during fetal development. Acquired hypopituitarism results from damage to the pituitary or hypothalamus that occurs during or following birth. It can be caused by a severe head injury, brain damage due to disease, radiation therapy, or a tumour.

The worldwide incidence of GHD in children has been estimated to be at least 1 in 10,000 live births and some individual countries have reported an incidence as high as 1 in 4,000 live births. A growth hormone deficient child usually shows a growth pattern of less than 2 inches a year. In many cases the child will grow normally until the age of 2 or 3 and then begin to show signs of delayed growth. Testing for growth hormone deficiency will occur when other possibilities of short stature have been ruled out. A weekly dose of up to

0.30 mg/kg of body weight divided into daily subcutaneous injections is recommended for GHD children.

In adults, deficiency of growth hormone can develop in the following situations; presence of a large pituitary tumour, after surgery or radiation therapy of pituitary tumour or other brain tumours, secondary to hypothalamic disorders and the continuation of childhood growth hormone deficiency into adulthood. The clinical features of adult GHD include; fatigue, muscle weakness, reduced exercise capacity, weight gain, increase in body fat and decrease in muscle mass, increase in LDL cholesterol and triglycerides and decrease in HDL cholesterol, increased risk for heart attack, heart failure and stroke, decrease in bone mass, anxiety and depression, especially lack of sense of well-being, social isolation and reduced energy. In the United States, an estimated total of 35,000 adults have GHD and approximately 6,000 new cases of GHD occur each year. For the average 70 kg man, the recommended dosage at the start of therapy is approximately 0.3 mg given as a daily subcutaneous injection. The dose can be increased, on the basis of individual requirements, to a maximum of 1.75 mg daily in patients younger than 35 years of age and to a maximum of 0.875 mg daily in patients older than 35 years. Lower doses may be needed to minimize the occurrence of adverse events, especially in older or overweight patients.

Prader-Willi Syndrome

Prader-Willi syndrome is a disorder of chromosome 15 characterised by hypotonia, hypogonadism, hyperphagia, cognitive impairment and difficult behaviour; the major medical concern being morbid obesity. Growth hormone is typically deficient, causing short stature, lack of pubertal growth spurt, and a high body fat ratio, even in those with normal weight. The need for GH therapy should be assessed in both children and adults. In children, if growth rate falls or height is below the third percentile, GH treatment should be considered. Growth hormone replacement helps to normalize the height and increases lean body mass; these both help with weight management. The usual weekly dose is 0.24 mg/kg of body weight; this is divided into 6 or 7 smaller doses over the course of the week.

Turner Syndrome

Turner syndrome occurs in approximately 1 in 2,500 live-born girls. It is due to abnormalities or absence of an X chromosome and is frequently associated with short stature, which can be ameliorated by GH treatment. Other features of Turner syndrome can include shortness of the neck and at times, webbing of the neck, cubitus valgus, shortness of fourth and fifth metacarpals and metatarsals, a shield shaped chest and primary

hypogonadism. Growth in height is variable in patients with Turner syndrome so the decision whether to treat with GH and the timing of such treatment is made on an individual basis. Often, treatment is initiated when a patient's height declines below the 5th percentile or when the standard deviation score decreases to less than 2 standard deviations below the mean. Treatment is often initiated with GH doses slightly higher than those used in treating GHD; a common starting dosage is 0.375 mg/kg per week divided into daily doses.

Chronic Renal Insufficiency

Chronic renal insufficiency (CRI) affects about 3,000 children in the United States. It manifests through a gradual and progressive loss of the ability of the kidneys to excrete wastes, concentrate urine, and conserve electrolytes. Approximately a third of children with chronic renal disease have abnormal growth partly because renal diseases disturb the metabolism of growth hormone. The corticosteroid hormones which are often used to treat the kidney disease can also retard growth. Kidney transplants can help a child start growing normally again, but most children do not make up the growth lost prior to transplantation. The age that the renal disease starts has more impact on growth retardation than the reduction in renal function (i.e. the younger the child when the disease starts, the more retarded is his or her growth). GH treatment can be given at a dosage of 0.35 mg/kg per week given six or seven times weekly.

Constitutional Delay of Growth

Constitutional delay of growth is characterized by normal prenatal growth followed by growth deceleration during infancy and childhood, and is reflected in declining height percentiles at this time. Between 3 years of age and late childhood, growth proceeds at a normal velocity. A period of pronounced growth deceleration can be observed immediately preceding the onset of puberty. Children with constitutional delay have later timing of puberty. At times, the combination of short stature accompanied and exaggerated by constitutional delay of growth and development in adolescents can cause sufficient psychosocial adolescent stress to warrant treatment with GH administered in the same manner and dosage as that used for treating GHD.

Cystic Fibrosis

Cystic Fibrosis (CF) is the most common lethal genetic disorder in America. An estimated 1000 individuals are born with Cystic Fibrosis each year in the United States. Cystic fibrosis causes dysfunction of the exocrine glands with increased viscosity of mucus

secretions, which leads to pulmonary disease, exocrine pancreatic insufficiency, and intestinal obstruction. Early diagnosis and treatment has significantly decreased mortality in children with CF. However, malnutrition and poor growth continue to be a significant problem. Poor weight gain, weight loss, and inadequate nutrition result from reduced energy intake, increased energy loss, and increased energy expenditure. It has been reported that 28% of persons with CF are below the 10th percentile for height and 34% are below the 10th percentile for weight. Studies have shown that GH therapy improves height velocity, weight velocity, lean body mass (LBM) and pulmonary function in patients with cystic fibrosis.

Skeletal Dysplasias

Skeletal dysplasias associated with short stature such as achondroplasia can be treated with GH. Achondroplasia is a genetic disorder, affecting the fibroblast growth factor receptor type III gene, which is evident at birth. It affects about one in every 20,000 births and it occurs in all races and in both sexes. During fetal development and childhood, cartilage normally develops into bone, except in a few places, such as the nose and the ears. In individuals with achondroplasia the rate at which cartilage cells in the growth plates of the long bones turn into bone is slow, leading to short bones and reduced height.

Achondroplasia is characterized by short stature, short limbs, proximal extremity (upper arm and thigh), head appears disproportionately large for body, skeletal (limb) abnormalities, abnormal hand appearance (trident hand) with persistent space between the long and ring fingers, marked kyphosis and lordosis (spine curvatures), waddling gait, bowed legs, prominent (conspicuous) forehead (frontal bossing), hypotonia and polyhydramnios (present when affected infant is born). GH has been approved to treat achondroplasia in some countries such as Japan and South Africa but does not yet have FDA approval.

Intrauterine Growth Retardation (IUGR) and Children of Small Gestational Age (SGA Children)

GH treatment can be beneficial in children with inter uterine growth retardation or infants who are small for gestational age (a condition also termed Russell-Silver syndrome). One definition of inter uterine growth retardation is a weight below the 10^{th} percentile for gestational age or a birth weight 2 standard deviations below the mean for gestational age. Studies have shown that those children who don't show catch-up growth can benefit from GH treatment.

The present invention resides in the surprising finding that co-administration of GH with FFA regulators ameliorates the deterioration of insulin sensitivity through prevention of lipolysis, has decreased oedemic effects in comparison with the GH therapy alone and exerts synergism to increase linear growth above that of GH alone.

The novel application disclosed in the invention provides a new method and a composition aimed at alleviating the conditions associated with GH therapy and enhancing the efficacy of the methods existing in the prior art. Moreover, the novel application disclosed in the invention provides the public with a beneficial alternative to the methods existing in the prior art.

Methods of treatment

The invention in broad terms is directed to the treatment or prophylaxis of consequences of growth hormone (GH) treatment. GH is commonly used to treat conditions resulting in short stature including but not restricted to growth hormone insufficiency, growth hormone deficiency, Intrauterine Growth Retardation (Silver-Russell Syndrome), skeletal abnormalities, chromosomal variations (Turner's syndrome, Down syndrome), or chronic kidney disease related growth retardation. GH treatment has been shown to contribute to a number of conditions, as described earlier, such as type 2 diabetes and hypertension. Such conditions have also been observed to extend beyond immediate GH treatment. The applicants established that such consequences can at least be mitigated, if not completely prevented, by administration of a FFA regulator, preferably in combination with the GH treatment. The addition of FFAs to GH corrects insulin sensitivity to either the pre-treatment state or to that of normal children. Where the adverse consequences of growth hormone treatment have not been observed as symptoms, the incidence of the consequences can at least be mitigated prophylactically.

Of particular advantage is that while the adverse effects of the GH therapy are alleviated, the growth increase effect of the GH is enhanced by the use of the FFA regulator. As a result, the combination treatment provides a useful method of treating the short stature condition (with administration of GH) while at the same time at least reducing some of the adverse consequences of the treatment.

In one aspect, this invention is a method of preventing or treating the adverse consequences of growth hormone treatment of a growth disorder in a mammal, especially a human which may be a child or an adolescent, comprising administering an effective amount

of a FFA regulator or a combination of FFA regulators prior to, in combination with or following the growth hormone treatment.

In a further aspect, this invention is a method of treating growth disorders in a mammal especially a human which may be a child or an adolescent, comprising administering an effective amount of a FFA regulator or a combination of FFA regulators in combination with the growth hormone treatment.

Examples of FFA regulators include the fibric acid derivative drugs (fibrates) and nicotinic acid analogues *e.g.* acipimox. Acipimox and the fibrates are hypolipidemic agents that efficiently lower serum triglyceride levels. Fibrates are known to effect this lowering of triglycerides through activation of the peroxisome proliferator-activated receptor-α (PPAR-α).

In a yet further aspect the present invention includes a prophylactic method of treating diabetes mellitus, dyslipidemia, hypertension and/or obesity in mammals suffering from growth disorders.

In a still other aspect the present invention provides for the use of growth hormone and a FFA regulator or a combination of FFA regulators in the preparation of a medicament or composition for treating growth disorders and /or preventing or treating the adverse consequences of growth hormone treatment.

Pharmaceutical composition

In general, compounds of this invention will be administered as pharmaceutical compositions by one of the following routes: oral, topical, systemic (*e.g.* transdermal, intranasal or by suppository), parenteral (*e.g.*intramuscular, subcutaneous or intravenous injection), by implantation and by infusion through such devices as osmotic pumps, transdermal patches and the like. Compositions may take the form of tablets, pills, capsules, semisolids, powders, sustained release formulation, solutions, suspensions, elixirs, aerosols or any other appropriate compositions; and may include pharmaceutically acceptable excipients. Suitable excipients are well known to persons of ordinary skill in the art, and they, and the methods of formulating the compositions, may be found in such standard references as Gennaro AR: *Remington: The Science and Practice of Pharmacy,* 20th Ed., Lippincott, Williams and Wilkins, Philadephia, PA (2000). Suitable liquid carriers, especially for injectable solutions include water, aqueous saline solution, aqueous dextrose solution and the like, with isotonic solutions being preferred for intravenous administration.

Compounds of this invention are also suitably administered by a sustained-release system. Suitable examples of sustained release compositions include semi-permeable polymer matrices in the form of shaped articles *e.g.* films or microcapsules. Sustained release matrices include polylactides (U.S. Pat. No. 3,773,919; EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, poly(2-hydroxyethyl methacrylate), ethylene vinyl acetate or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include a liposomally entrapped compound. Liposomes containing the compound are prepared by methods known per se: DE 3,218,121; EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Apln. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545 and EP 102,324.

Compounds of this invention may also be PEGylated to increase their lifetime in *vivo*, based on *e.g.* the conjugate technology described in WO 95/32003.

Preferably, the FFA regulator will be administered orally and Growth Hormone by subcutaneous injection.

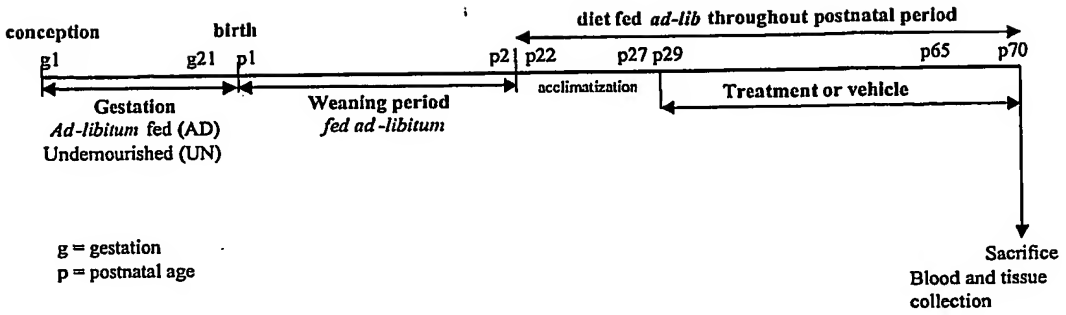
The foregoing describes the invention including preferred forms thereof. Alterations and medications that would be apparent to the skilled person are intended to be included within the spirit and scope of the invention disclosed.

PHARMACOLOGICAL STUDY 1

A study to assess the effectiveness of a combination therapy comprising GH and FFA regulators in improving linear growth and reducing metabolic abnormalities associated with GH therapy.

Study design

This study utilised a well-characterised rodent model of short stature due to fetal growth retardation with an inherent tendency to insulin resistance. A schematic of the overall experimental design is presented below.



Test Groups- 10 animals per group

Undernourished (UN)	*Ad Libitum* (AD)
Control	Control
5mg/kg GH	5mg/kg GH
5mg/kg GH + fenofibrate	5mg/kg GH + fenofibrate
5mg/kg GH + acipimox	5mg/kg GH + acipimox

Experimental procedure - methods and analytical procedures

Animal model

The rodent model of maternal undernutrition used to induce SGA was initially characterised in the Liggins Institute, Faculty of Medical and Health Sciences, University of Auckland by Woodall *et al.* (1996). This model has since been published in several international peer-reviewed journals (Woodall *et al.* 1996, 1998; Vickers *et al.* 2000, 2001).

This experimental approach to induce SGA results in 30-35% growth retardation in 22-day old fetuses and persistent postnatal growth failure and no evidence of catch-up growth until at least 90 days of age. These animals develop hypertension, insulin resistance and truncal obesity as adults.

Animal protocol to generate SGA offspring

Virgin Wistar rats (age 75-100 days) were time mated using a rat estrous cycle monitor (Fine Science Tools INC., North Vancouver, BC, Canada) to assess the stage of estrous of the animals prior to introducing the male. Day 1 of pregnancy was determined by the presence of spermatozoa in a vaginal smear. After confirmation of mating, rats were housed individually in standard rat cages containing wood shavings as bedding with free

access to water. The animal room was maintained at 25°C with a 12 hour light: 12 hour dark cycle. Dams were randomly assigned to receive food either *ad-libitum* (AD group and dams for cross fostering) or to receive 30% of *ad-libitum* (UN-group, determined by measuring food intake on the previous day of an *ad-libitum* fed dam). The diet composition was protein 18%, fat 4%, fibre 3%, ash 7% and carbohydrate 58% (Diet 86, Skellerup Stock Foods, Auckland, New Zealand). Food intake and body weight was recorded daily. Following birth, UN offspring were cross fostered onto *ad-libitum* fed mothers. Cross fostering is necessary due to lactational insufficiency in restricted fed dams. Litter size was adjusted to 8 pups per litter to assure adequate and standardised nutrition. Body weight of all pups was recorded daily. At weaning (age 21 days) pups were sexed, weight-matched and housed in pairs in standard cages. All animals were fed *ad-libitum* for the remainder of the study. Dams were sacrificed by CO_2 asphyxiation and excess pups by decapitation.

In this experiment, male offspring only were used.

The use of power calculations determined that group sizes of 10 were necessary to demonstrate statistically significant differences anticipated in body length and fasting insulin concentration.

Test compounds

Recombinant bovine growth hormone (rbGH)

Many studies in rodents utilize treatment with human GH (hGH) due to its relative ease of availability for experimental use. However, hGH possesses both lactogenic and somatogenic properties in the rat due to hGH binding to both prolactin receptors and GH receptors. This has been clearly documented in binding studies using hGH, bGH, oPRL and rat growth hormone (rGH) and rat prolactin (rPRL). Rat hepatocytes contain two types of binding sites that bind hGH. The first, somatogenic binding sites, are specific for the growth-promoting hormones bGH and rGH. The second, lactogenic, are specific for lactogenic hormones, oPRL and rPRL. Human GH has been shown to bind to both sites (Ranke et al., 1976).

Recombinant rat GH was not available in sufficient quantities for large-scale animal experiments. Therefore bGH, a pure somatogen in the rat and an agent which is not a ligand for the rat prolactin receptor (Yamada et al, 1984), was used in the studies.

Fibrates

Fenofibrate belongs to the class of fibrates (fibric acid derivative drugs). Fibrates are hypolipidemic agents that efficiently lower serum triglyceride levels through mediation of the peroxisome proliferator-activated receptor-α (PPAR-α). In addition, fibrates are known to lower serum cholesterol levels.

Fenofibrate was administered by daily oral gavage at a dosage of 30mg/kg body weight /day.

Acipimox

Acipimox is a potent long-acting nicotinic acid (NA) analog. As a hypolipidaemic agent acipimox reduces serum concentrations of triglycerides and non-esterified fatty acids. Acipimox has been shown to partially prevent GH induced insulin resistance by inhibition of lipolysis (Segerlantz *et al.* 2001).

Acipimox (Pharmacia) was administered by daily oral gavage at a dose of 20 mg/kg body weight/day (Blachere *et al.* 2001).

Observations

Food consumption

Food intake was measured on a daily basis. Relative food intake per rat (grams consumed per gram body weight per day) was calculated using the amount of food given to and the amount of uneaten food left by each pair in each group.

Water Consumption

Water consumption was calculated daily by weighing water bottles at the same time on each day of the study.

Bodyweight

Rats were weighed each day at 0900 h.

Body length

Body lengths (nose-anus and nose-tail) and bone length (tibial, femoral length) was assessed post-mortem using peripheral quantitative computed tomography (pqCT) analysis.

Blood pressure

Systolic and diastolic blood pressure and heart rate were recorded by tail cuff plethysmography according to the manufacturer's instructions (Blood pressure analyser

IITC, Life Science, Woodland Hills, CA, USA). Rats were restrained in a clear plastic tube in a heated room (25-28°C). After 10-15 minutes acclimatisation the cuff was placed on the tail and inflated to 240mmHg. Pulses were recorded during deflation at a rate of 3mmHg/sec and reappearance of a pulse was used to determine systolic blood pressure. A minimum of 3 clear systolic blood pressure recordings were taken per animal. Previous observations indicate that the coefficient of variation for repeated measurements is <5%.

Biochemistry and endocrine analyses

Blood samples were collected following overnight fast. Samples were collected from the tail vein and at termination following decapitation under halothane anaesthetic. Blood samples were collected into heparinised tubes and centrifuged for harvesting of plasma. Blood samples were then analysed for insulin, glucose, FFAs, leptin, IGF-I, glycerol, triglycerides, cholesterol, corticosterone, markers of hepatic function (ALT, AST, ALP), and for markers of protein synthesis. IGFBPs will be evaluated by Western blot.

Plasma FFAs, triglycerides and glycerol were measured by diagnostic kit (Boehringer-Mannheim #1383175 and Sigma #337 respectively). Plasma leptin, insulin were measured using commercially available kits (Linco, St Charles, MO, US). Plasma IGF-I was measured by RIA as described previously (Vickers et al., 2000). Plasma Glucose concentrations were measured using a YSI Glucose Analyzer (Model 2300, Yellow Springs Instrument Co., Yellow Springs, OH, US). All other plasma analytes (liver enzymes, electrolytes etc) were measured by a BM/Hitachi 737 analyser by Agriquality Laboratory Services (Auckland, New Zealand).

Tissue studies

At termination animals were sacrificed by decapitation under halothane anaesthesia. Tissues (heart, liver, muscle and adipose (subcutaneous and visceral)) were collected, weighed and snap frozen in liquid nitrogen for subsequent analysis. An aliquot of liver tissue was also frozen at –20°C for examining the growth hormone receptor using ligand-binding analysis.

Specific binding of ^{125}I-bGH to hepatic microsomal membrane preparations

Microsomal membrane preparations were prepared as previously described (Breier *et al.* 1988) and receptor assays were performed as previously validated for the rat liver (Singh *et al.* 1992). Radiolabelled rbGH was obtained using a modified lactoperoxidase method to

obtain a specific activity of 70-90 μCi/μg (Thorell and Johansson 1971). Membrane preparations (100μl) were incubated with approximately 40000cpm ^{125}I-bGH in a total incubation volume of 500μl. Non-specific binding was determined by addition of 1000ng of unlabelled bGH. Equilibrium conditions were reached and specific ^{125}I-bGH binding was reversed by the addition of excess unlabelled bGH. The method of Lowry (Lowry *et al.* 1951) was used to determine MMP protein concentrations. Specific ^{125}-I-bGH binding was corrected for the protein content of the MMP.

Data analysis

Data was analysed using multiple regression analysis or factorial ANOVA / ANCOVA with *post hoc* correction (prenatal influences and postnatal treatment effects) where appropriate. Growth rates were compared using multiple regression analysis. Changes in body length before and after treatment have been compared using repeated measures ANOVA.

Previous data provided the basis of power calculations for the proposed studies (assuming α=0.05). For insulin sensitivity, an n of 10 will detect with a power of 80% a change of 0.2 and at 95% a change of 0.26ng/ml with an SD of 0.15ng/ml. For body length, an n of 10 will detect with a power of 80% a change of 6.88mm and at 95% a change of 7.97mm with an SD of 5.2mm

5 Bibliography

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WE CLAIM

1. A method of preventing or treating the adverse consequences of growth hormone treatment of a growth disorder in a mammal, comprising administering an effective amount of a FFA regulator or a combination of FFA regulators prior to, in combination with or following the growth hormone treatment.

2. The method of claim 1 wherein the adverse consequences of growth hormone treatment are insulin resistance and/or secondary hyperinsulinemia.

3. The method of claim 1 wherein the adverse consequences comprise a development of diabetes mellitus, dyslipidemia, hypertension and/or obesity.

4. The method of claim 1 wherein the adverse consequences comprise oedema.

5. A method of treating growth disorders in a mammal comprising administering an effective amount of FFA regulator or a combination of FFA regulators in combination with the growth hormone treatment.

6. The method of either one of claims 1 or 5 wherein the mammal is human.

7. Method of increasing the growth promoting effects of GH therapy, the said method comprising administering an effective amount of FFA regulator or a combination of FFA regulators in combination with the growth hormone treatment.

8. The method of either one of claims 1 or 5 wherein the mammal is a child or an adolescent.

9. The method of either one of claims 1 or 5 wherein the growth disorder is selected from a group consisting of a growth hormone insufficiency, growth hormone deficiency, Intrauterine Growth Retardation, prematurity, skeletal abnormalities, chromosomal variations (Turner's syndrome, Down syndrome), chronic kidney disease related growth retardation or any other condition resulting in short stature.

10. The method of either one of claims 1 or 5 wherein the FFA regulators is a fibric acid or nicotinic acid derivative.

11. The method of either of claims 1 or 5 wherein the method of administration of the GH, FFAs or GH in combination with FFAs is oral, topical, systemic, parenteral, by implantation or by infusion.

12. The method of either of one of claim 1 or 5 wherein the GH is administered by subcutaneous injection.

13. The method of either one of claims 1 or 5 wherein the FFA regulator or a combination of FFA regulators is administered orally.

14. A composition or medicament for treating growth disorders and /or preventing or treating the adverse consequences of growth hormone treatment including GH and a FFA regulator or a combination of FFA regulators.

15. A composition according to claim 14 wherein the composition or medicament includes suitable pharmaceutical carriers and/or excipients for the growth hormone and FFA regulator or a combination of FFA regulators.

16. The use of growth hormone and a FFA regulator or a combination of FFA regulators in the preparation of a medicament or composition for treating growth disorders and /or preventing or treating the adverse consequences of growth hormone treatment

17. A composition or medicament according to claim 14 wherein the composition or medicament is adapted for sequential administration.

18. A composition or medicament according to either one of claims wherein the FFA is a fibric acid derivative or a nicotinic acid derivative.

19. A method of treatment or prophylaxis substantially as herein described with reference to any of the Figures.

20. A composition or medicament substantially as herein described with reference to any one of the examples.

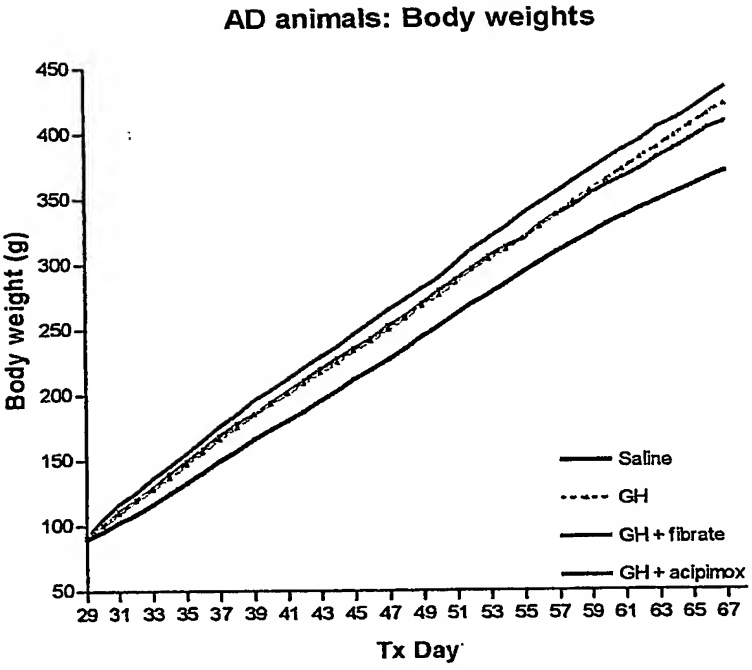
AD animals: Body weights
Body weight (g)
450
400
350
300
250
200
150
100
50
29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67
Tx Day
Saline
GH
GH + fibrate
GH + acipimox

FIG. 1A

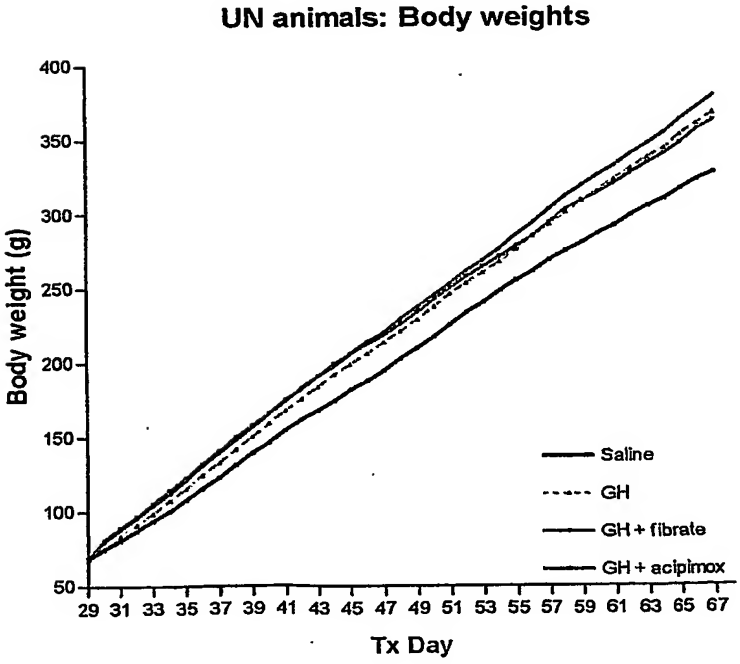
UN animals: Body weights
Body weight (g)
400
350
300
250
200
150
100
50
29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67
Tx Day
Saline
GH
GH + fibrate
GH + acipimox

FIG. 1B

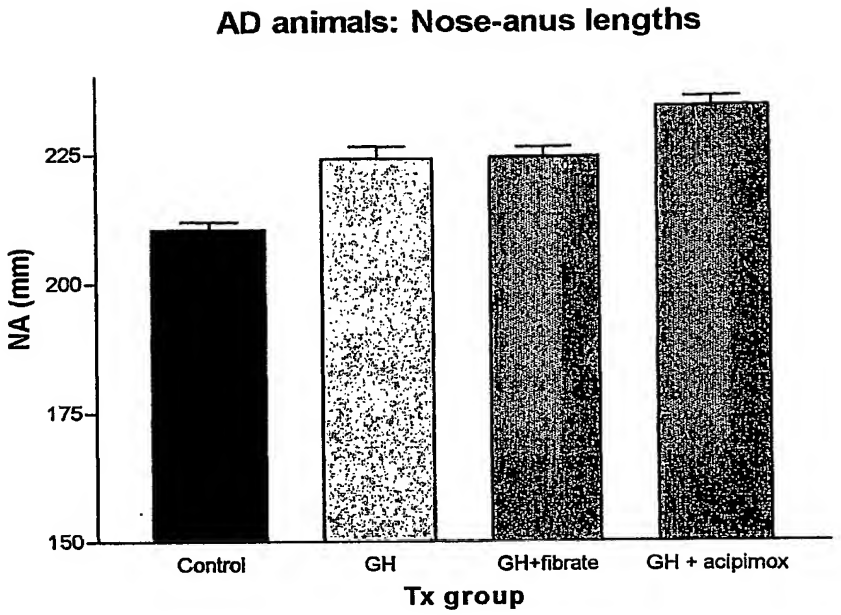
AD animals: Nose-anus lengths
225
200
175
150
NA (mm)
Control
GH
GH+fibrate
GH + acipimox
Tx group

FIG. 2A

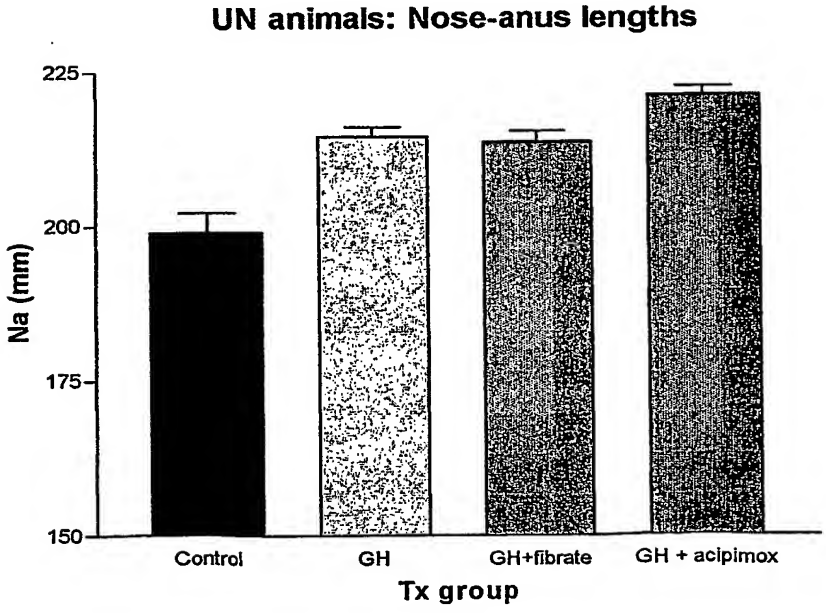
UN animals: Nose-anus lengths
225
200
175
150
Na (mm)
Control
GH
GH+fibrate
GH + acipimox
Tx group

FIG. 2B

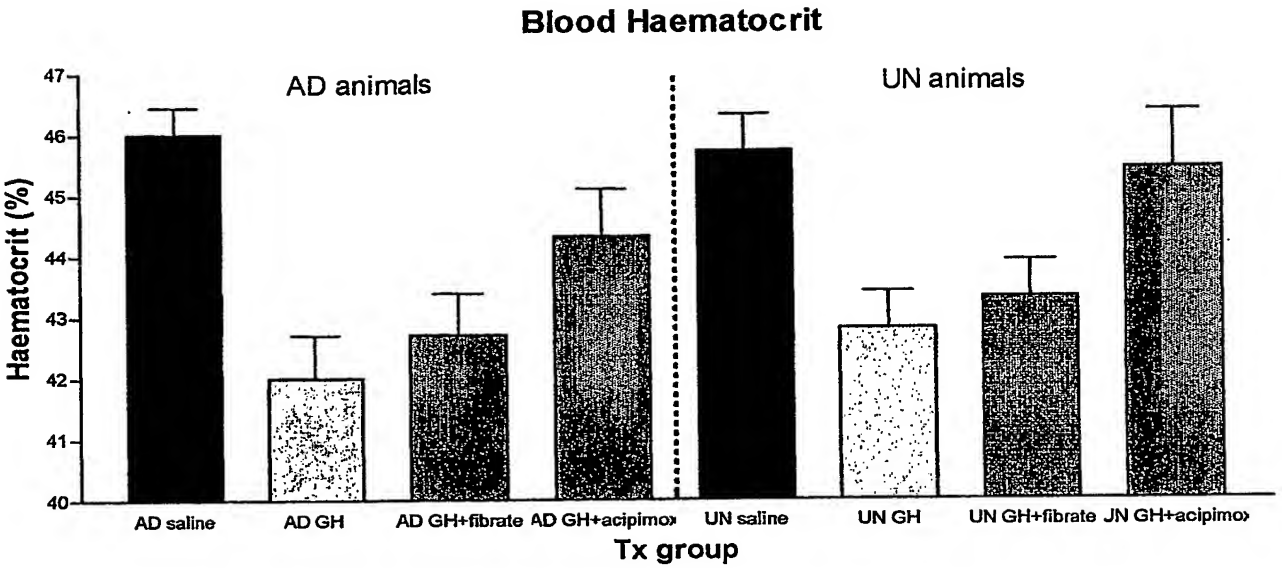
Blood Haematocrit
AD animals
UN animals
Haematocrit (%)
47
46
45
44
43
42
41
40
AD saline
AD GH
AD GH+fibrate
AD GH+acipimox
UN saline
UN GH
UN GH+fibrate
JN GH+acipimox
Tx group

FIG. 3

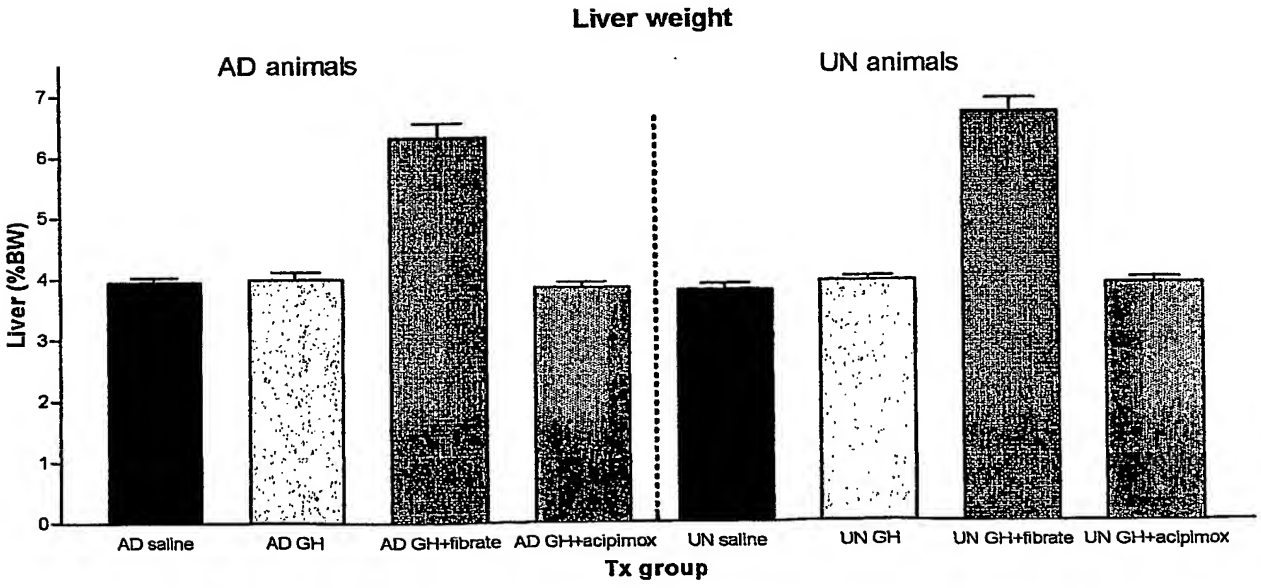
Liver weight
AD animals
UN animals
Liver (%BW)
7
6
5
4
3
2
1
0
AD saline
AD GH
AD GH+fibrate
AD GH+acipimox
UN saline
UN GH
UN GH+fibrate
UN GH+acipimox
Tx group

FIG. 4

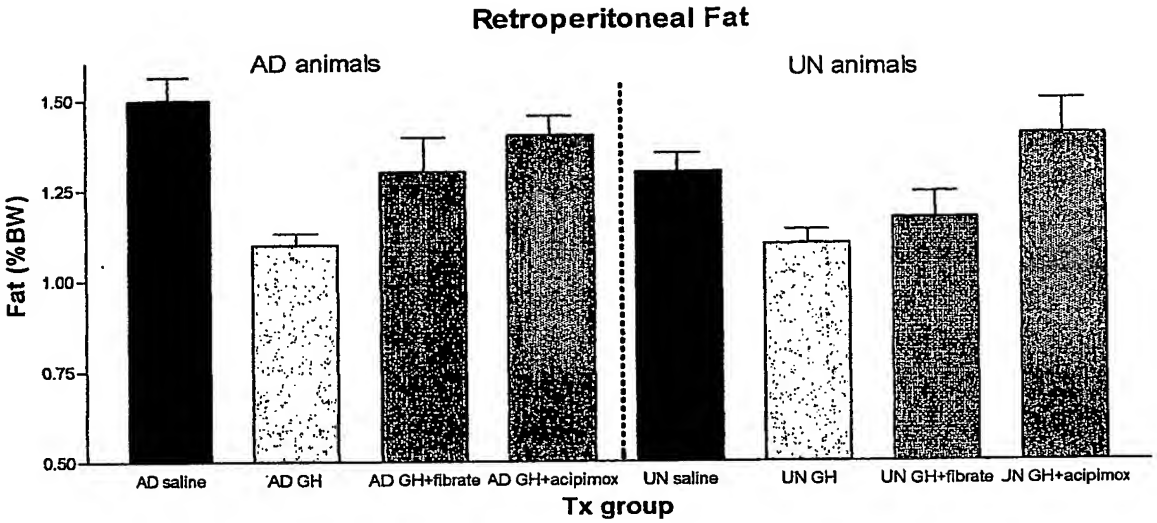
Retroperitoneal Fat
AD animals
UN animals
Fat (%BW)
1.50
1.25
1.00
0.75
0.50
AD saline
AD GH
AD GH+fibrate
AD GH+acipimox
UN saline
UN GH
UN GH+fibrate
JN GH+acipimox
Tx group

FIG. 5

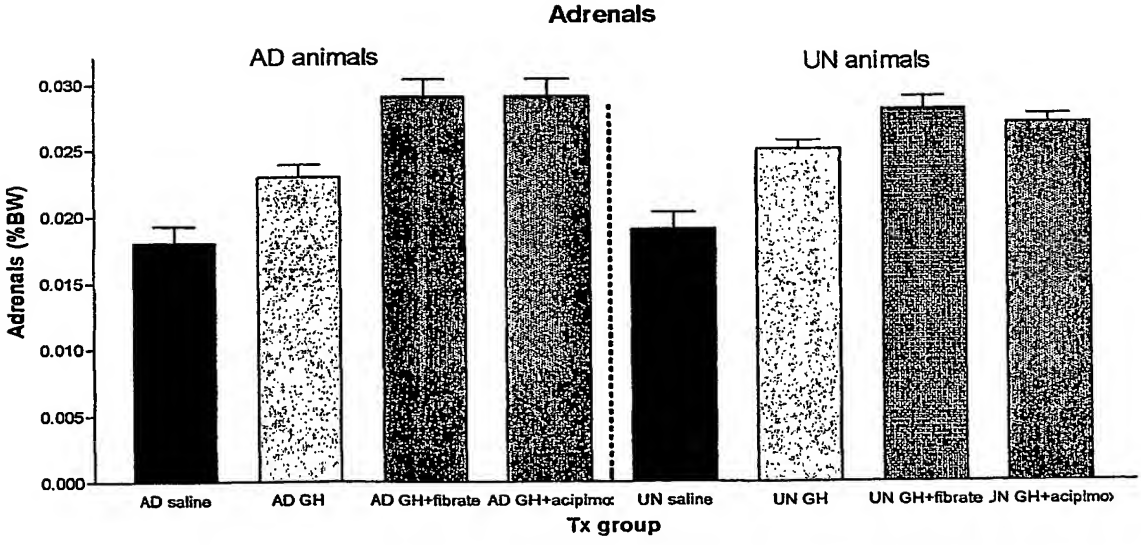
Adrenals
AD animals
UN animals
Adrenals (%BW)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
AD saline
AD GH
AD GH+fibrate
AD GH+acipimox
UN saline
UN GH
UN GH+fibrate
JN GH+acipimox
Tx group

FIG. 6

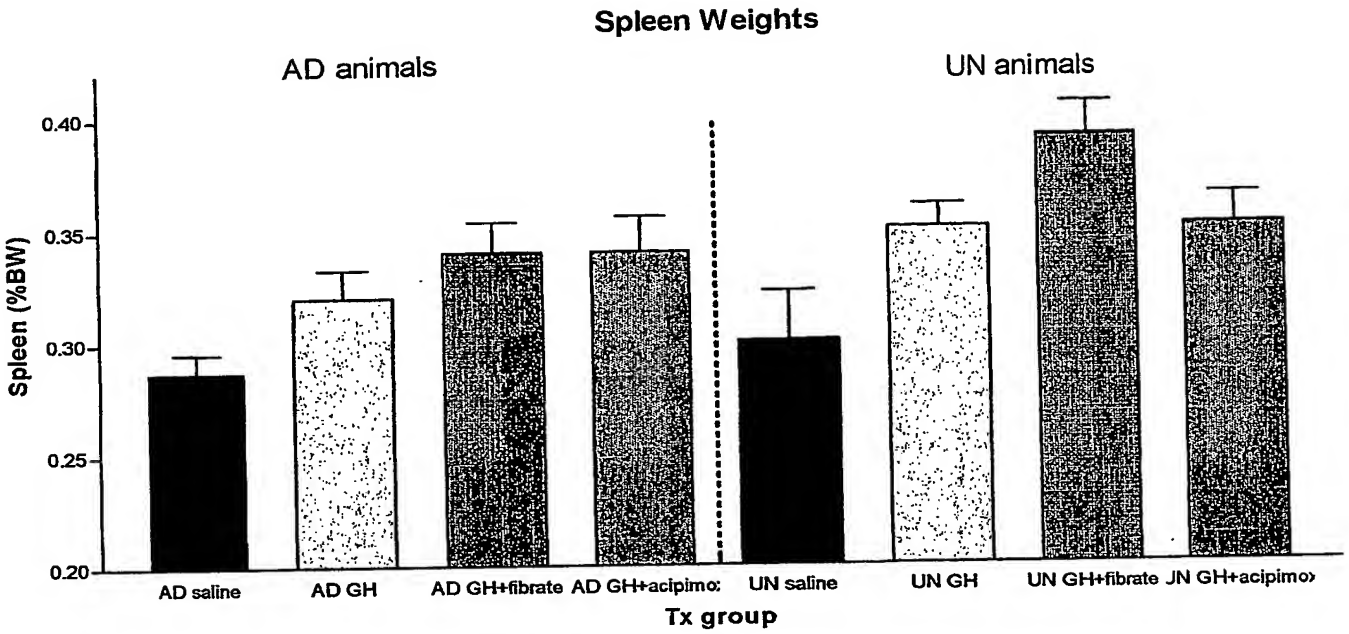
Spleen Weights
AD animals
UN animals
Spleen (%BW)
0.40
0.35
0.30
0.25
0.20
AD saline
AD GH
AD GH+fibrate
AD GH+acipimo:
UN saline
UN GH
UN GH+fibrate
JN GH+acipimo›
Tx group

FIG. 7